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14. ABSTRACT

We have developed a 60-gene expression profile that may identify tumors with a BRCAness phenotype which is characterized by heightened sensitivity to chemotherapy (platinum and PARP inhibitors) and improved overall survival. We found that our profile can track diverse molecular mechanisms that cause defective homologous recombination (HR) in the absence of BRCA1 or BRCA2 mutations. Furthermore, we found that the BRCAness profile was associated with overall survival and response to platinum chemotherapy in vitro and in vivo using several metrics of clinical platinum sensitivity including disease free survival, achievement of complete response after first line therapy and percent progression free survival at 4 months, 6 and 18 months. Multivariate analysis and independent validation of the profile in patients from other institutions demonstrated that it maintained an independent association with disease free and overall survival. Our current studies are focusing on assessing the correlation of the profile with PARP inhibitor responsiveness, and evaluating the reproducibility of the profile using the DASL mRNA assay in formalin fixed, paraffin-embedded ovarian cancer specimens.

15. SUBJECT TERMS
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INTRODUCTION

It is well established that hereditary ovarian cancer patients associated with germline BRCA-1 or BRCA-2 mutations exhibit two important clinical characteristics: i) heightened sensitivity to platinum analogues and PARP inhibitors (presumably due to an underlying defect in homologous recombination (HR)) and ii) improved survival compared to their sporadic counterparts. Importantly, certain sporadic tumors may also have abnormalities in HR (in the absence of germline BRCA mutations) and thus behave similarly to those with BRCA germline mutation. Such sporadic tumors are referred to as having a "BRCAness" phenotype that is characterized by heightened sensitivity to chemotherapy (platinum and PARP inhibitors) and improved overall survival.

However, prospective identification of sporadic tumors with a BRCAness phenotype is challenging. This is because there are several molecular mechanisms that may underlie defective HR in a sporadic tumor in the absence of germline BRCA mutations. These mechanisms include epigenetic hypermethylation of the BRCA-1 gene promoter, somatic mutations of BRCA1 or BRCA2, loss of function mutations or epigenetic inactivation of other HR genes, and amplification of genes that inactivate BRCA gene function (e.g. EMSY which inactivates BRCA2). Given the heterogeneous mechanism(s) by which an ovarian cancer cell might harbor a BRCAness phenotype, we reasoned that a broad-based approach like gene expression profiling, which makes few assumptions about mechanism might have the highest chance of capturing sporadic tumors with a BRCAness phenotype. In this regard, we have developed a 60-gene expression profile that may identify tumors with a BRCAness phenotype. This profile designates tumors as BRCA-like (BL) or non-BRCA-like (NBL) corresponding to tumors predicted to have a BRCAness phenotype (BL tumors) or not (NBL tumors).

In this grant we have proposed a series of studies to evaluate the ability of this profile to identify tumors with defects in homologous recombination, increased sensitivity to platinum and PARP inhibitors in vitro, clinical response to platinum and survival in patients with sporadic ovarian cancer and to identify compounds that can reverse PARP inhibitor resistance in vitro. In this first annual report, we present the results of our studies thus far.

BODY

Aim 1: Determine whether the BRCAness gene expression profile is capable of prospectively identifying sporadic patients whose tumors exhibit defects in homologous recombination and increased sensitivity to platinum and PARP inhibitors in vitro **(months 1-60)**

Thus far, we have evaluated the ability of the BRCAness gene expression profile to identify defects in homologous recombination in several cellular systems. We have also evaluated the association of our BRCAness profile with platinum sensitivity in vitro.

The protein kinase ATM (ataxia-telangiectasia mutated) is a damage-response kinase that is responsible for the phosphorylation of Fanconi-Anemia (FA) proteins, which play an important role in DNA repair through homologous recombination (HR) (1). We have accessed gene expression (2) data of cells that were stably knocked down for ATM gene using retroviral vectors expressing the corresponding short hairpin RNA (shRNA). Gene expression data from control cells were also accessed. Cells that were stably knocked down for ATM gene had an impaired DNA damage response and an inactive HR DNA repair as opposed to control cells. Therefore cells knocked down for ATM and control cells afforded us the opportunity to assess whether the BRCAness profile could track cells with a defect in HR (in this case cells knocked down for ATM).

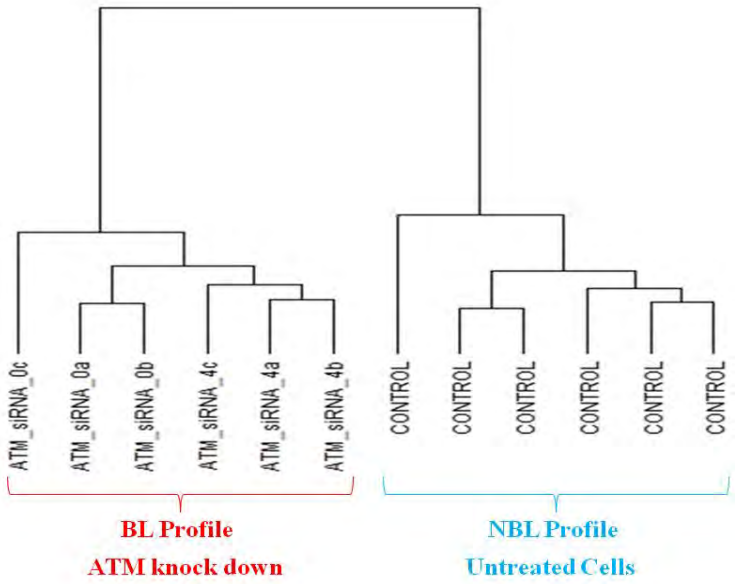

Figure 1. BRCAness profile distinguishes between ATM knock down and control cells

As shown in Figure 1 above, hierarchical clustering based on the expression pattern of the 60 genes of the BRCAness profile showed that the BRCAness profile could accurately distinguish between ATM knock down (HR deficient) cells and control (HR efficient) cells in 12 specimens. Specifically, six of six specimens with ATM knock down had the BL signature and six of six control specimens had the NBL signature (Fisher's exact two sided p=0.002).

We also evaluated the ability of the BRCAness profile to distinguish between cells that were knocked down for BRCA1 (via adenoviral BRCA1-RNAi) or control cells. Cells that were stably knocked down for BRCA1 gene had an inactive HR DNA repair as opposed to control cells and thus provided the opportunity to assess whether our profile could track a defect in HR in the absence of BRCA1 or BRCA2 mutations. Again, we accessed gene expression profiling data from BRCA1 knock down and control cells (3).

As shown in Figure 2 below, hierarchical clustering based on the expression pattern of the 60 genes of the BRCAness profile showed that the BRCAness profile could accurately distinguish between BRCA1 knock down (HR deficient) cells and control (HR efficient) cells in 6 specimens. Specifically, two of two specimens with BRCA1 knock down had the BL signature and four of four control specimens had the NBL signature (Fisher's exact two sided p=0.067).

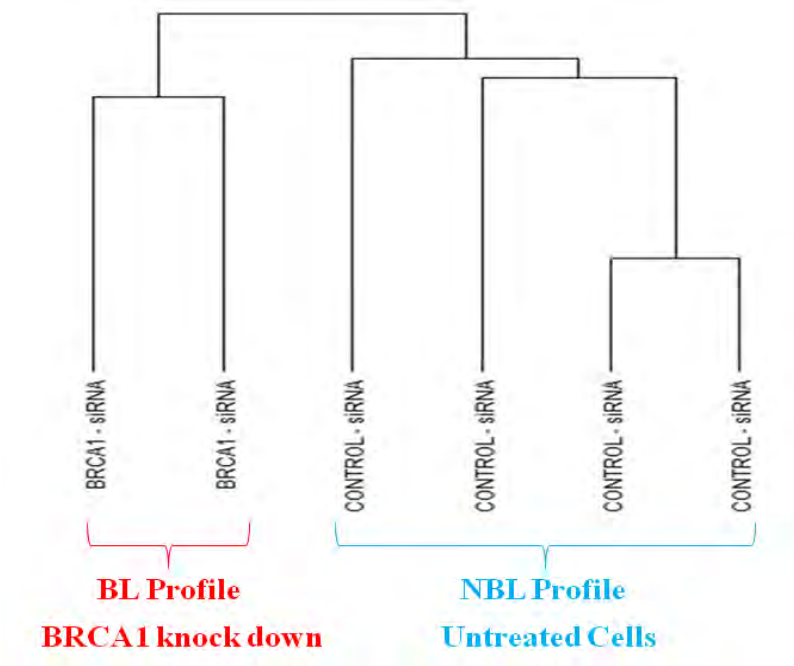

Figure 2. BRCAness profile distinguishes between BRCA1 knock down and control cells

The aforementioned findings (a+b) are very important because it is the first time that we show that our profile may be able to track defects in HR (i.e. ATM or BRCA1 knock down) in the absence of BRCA1 or BRCA2 mutations. As mentioned above, deficiency in homologous recombination is an important feature of BRCAness. Therefore, the ability of our profile to track diverse molecular mechanisms that cause defective HR represents an important validation step in terms of its ability to prospectively detect sporadic tumors with a BRCAness phenotype.

We have evaluated the association of our BRCAness profile with cisplatin sensitivity in vitro using the National Cancer Institute (NCI) 60 anticancer drug screen (NCI60) tumor cell lines. We accessed the gene expression profiling data as well as the cisplatin response parameters TGI (total growth inhibition) and LC50 (50% lethal concentration) for each of the NCI60 cell lines (4, 5). We then applied our BRCAness profile and identified which cell lines had a BRCAlike (BL) profile or a nonBRCAlike (NBL) profile. Cell lines with the BL profile had a lower TGI (16.9 vs 38.7μg/ml, p=0.016) and LC50 (288 vs 465 μg/ml, p=0.0005) compared to the cell lines with the NBL profile respectively, suggesting that the BL profile correlated with increased responsiveness (sensitivity) to cisplatin (Figure 3).

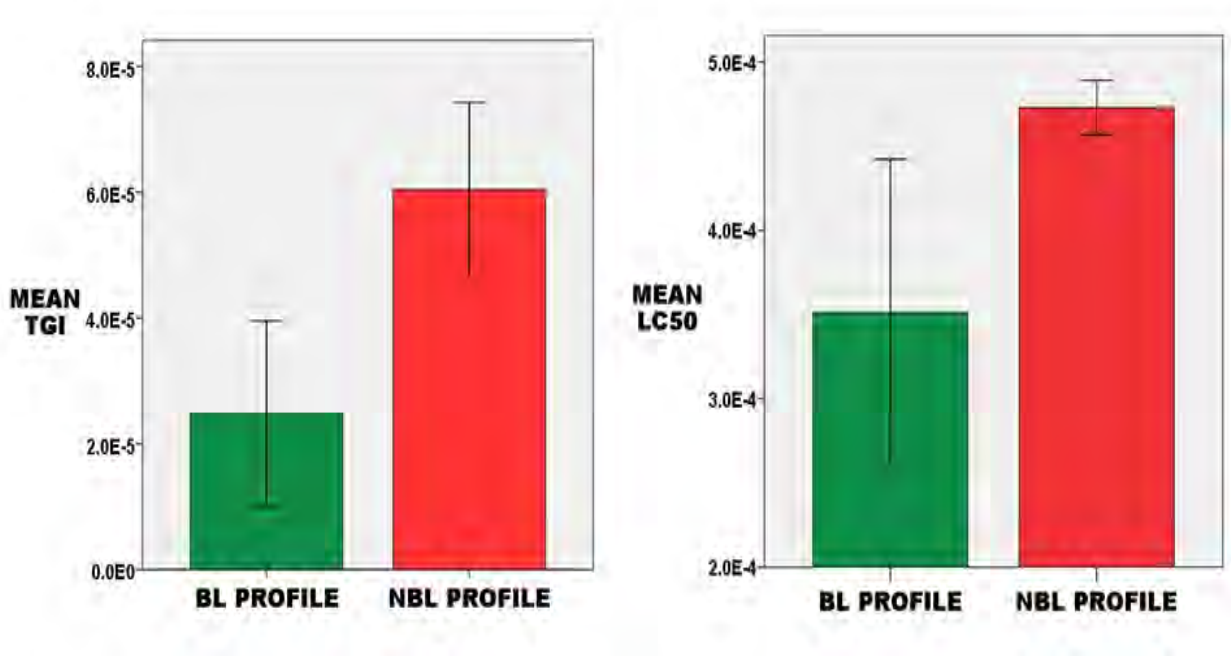

Figure 3. Association between BRCAness profile (BL vs NBL) with cisplatin sensitivity (as expressed by TGI and LC50) in the NCI60 tumor cell lines

Aim 2. Determine whether the BRCAness gene expression profile is associated with clinical response to platinum and survival in patients with sporadic ovarian cancer **(months 1-60)**

In order to evaluate whether the BRCAness gene expression profile is associated with clinical response to platinum and survival in patients with sporadic ovarian cancer we used a patient cohort that included 70 patients treated at Beth Israel Deaconess Medical Center, Memorial Sloan-Kettering Medical Center, and Cedars-Sinai Medical Center. All these patients had undergone exploratory laparotomy for diagnosis, staging, and debulking followed by first-line platinum-based chemotherapy. Standard post-chemotherapy surveillance included serial physical examination, serum CA-125 level, and computed tomography scanning as clinically indicated. This 70-patient cohort included two 35-patient cohorts as follows: The first cohort included tumor samples from 35 patients with invasive EOC who underwent sequencing for germline mutation (by using DNA obtained from peripheral-blood leukocytes) and did not harbor germline BRCA1 or BRCA2 mutations. The second cohort included 35 patients who did not undergo genetic testing but who were enriched for sporadic disease on the basis of the following characteristics: no family history of ovarian cancer, no family history of breast cancer younger than 50 years of age, no family history of more than one breast cancer at any age, and not of Ashkenazi Jewish ethnicity.

Total RNA was isolated from patient tumor samples using Trizol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. cDNA synthesis and hybridization on oligonucleotide microarrays (U133 Plus 2.0 Array GeneChip, Affymetrix, Inc., Santa Clara, CA) containing approximately 54,700 transcripts were carried out using standard protocols. Microarray experiments were performed at the Dana Farber Cancer Institute Microarray Core Facility (http://chip.dfci.harvard.edu/). Raw data were processed using Robust Multi-Array (RMA) analysis. All raw microarray data have been deposited in GEO (Gene Expression Omnibus - GSE19829). These data have been published in Journal of Clinical Oncology (6).

The clinical and pathologic characteristics of all 70 patients are listed in (Supplement Table 1 included in the Appendix). Overall, 20 (29%) of the 70-patient cohort demonstrated the BL profile (eight of 35 in the sequenced group, and 12 of 35 in the non-sequenced group). As listed in Table 1 below, there were no differences in age, stage, grade, histology, or debulking status between the BL and the NBL signature groups. The ability to achieve a clinical remission for the BL and NBL groups was 90% compared with 74%, although this did not reach statistical significance (two-sided Fisher's exact P=0.2).

Characteristic		NBL Profile (n=50)		BL Profile (n=20)		p value
		No.	%	No.	%	
Age	Median (range)	61 (39-89)		59.25 (44-80)		0.55
Grade	1-2	9	18	1	5	0.477
	3	41	82	19	95	
Histology	Serous	46	92	19	95	0.412
	Clear Cell	1	2	1	5	
	Endometrioid	3	6	0	0	
Stage	2	3	6	0	0	0.479
	3	41	82	16	80	
	4	6	12	4	20	
Debulking Status	Optimal	33	67.3	16	80	0.386
	Suboptimal	16	32.7	4	20	
Achievement of CR after first-line therapy		74%		90%		0.2

Table 1. Association of BRCAness profile with clinical characteristics and remission status after first line therapy

For the entire 70-patient cohort, the BRCAness profile was capable of discriminating between long and short median disease free survival (DFS); the patients with BL and NBL profiles had median DFS times of 34 months and 15 months, respectively (log-rank P = 0.013; Figure 4). In addition, the percentages of patients who were disease free at 4 months for the BL and NBL groups were 90% and 64% (P = 0.04), respectively; at 6 months, percentages were 85% and 60%, respectively (P = 0.053); and at 18 months, percentages were 65% and 29%, respectively (P=0.007).

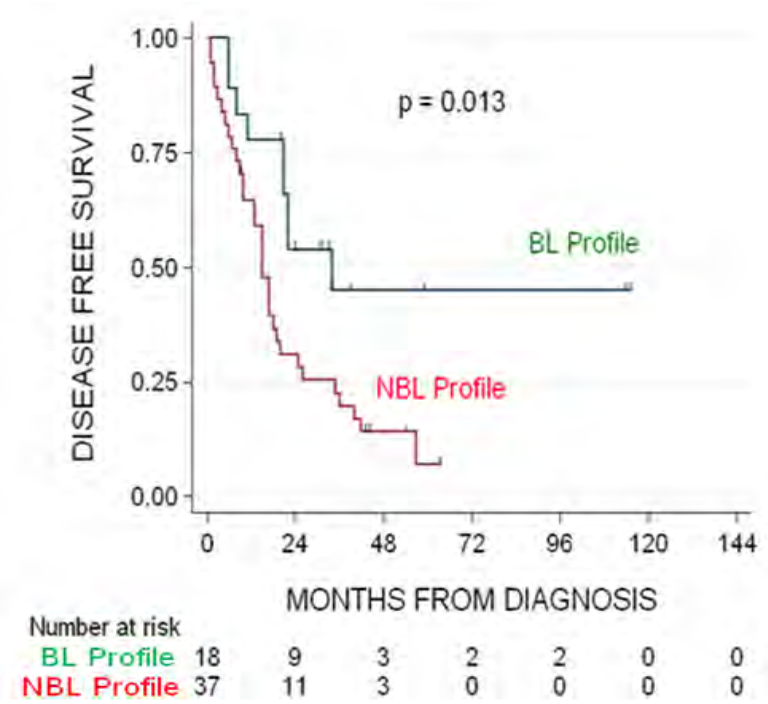

Figure 4. Association of BRCAness profile with DFS. Median DFS for BL and NBL patients was 34 and 15 months, respectively (*P* = 0.013).

Furthermore, the BRCAness profile distinguished between long and short median overall survival (OS), as the patients in the BL and NBL groups had median OS times of 72 and 41 months, respectively (log-rank P = 0.006; Figure 5). Similar findings were observed when applying the profile separately to the group of 35 sequenced patients who had undergone germline mutation testing and who were found to have wild-type BRCA1 and BRCA2 genes or to the group of the 35 non-sequenced patients enriched for sporadic disease on the basis of clinical characteristics, as previously described.

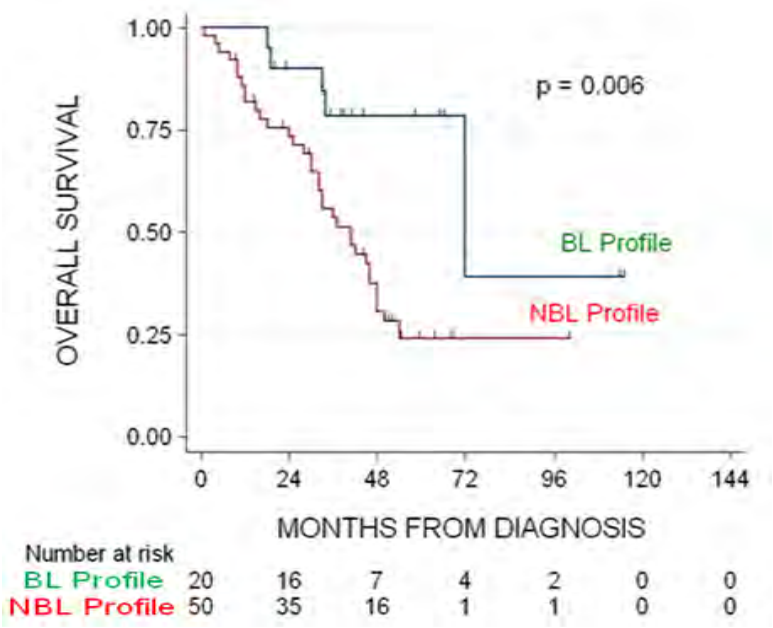

Figure 5. Association of BRCAness profile with OS. Median OS for BL and NBL patients was 72 and 41 months, respectively (*P* = 0.006).

In univariate analysis, the hazard ratio for recurrence (NBL v BL group) was 2.47 (P=0.018; 95% CI, 1.17 to 5.2), and the hazard ratio for death (NBL v BL group) was 3.29 (P=0.009; 95% CI, 1.34 to 8.09). Multivariate analysis, which included the BRCAness profile, age, stage, grade, histology, and debulking status, demonstrated that the profile maintained an independent association with DFS and OS (Table 2). The hazard ratio for recurrence (NBL v BL group) was 2.65 (P=0.016; 95% CI, 1.2 to 5.86), and the hazard ratio for death (NBL v BL group) was 3.39 (P=0.009; 95% CI, 1.35 to 8.5).

Factor	Univariate P Value		Multivariate P Value	
	DFS	OS	DFS	OS
Age	0.44	0.35	0.96	0.8
Grade	0.9	0.34	0.87	0.32
Histology	0.97	0.61	0.9	0.96
Stage	0.43	0.54	0.4	0.61
Debulking Status	0.39	**0.05 (HR=1.84)** [a]	0.53	0.67
NBL/BL profile	**0.018 (HR=2.47)**	**0.009 (HR=3.29)**	**0.016 (HR=2.65)**	**0.009 (HR=3.39)**

Table 2. Predictive value of BRCAness profile adjusted for Grade, Age, Histology, Stage and Debulking

Besides the aforementioned 70-patient cohort, we also applied our BRCAness profile in another dataset that was also published by us (7). This dataset included 47 patients with ovarian cancer treated at Duke University for whom data were available on grade, stage, debulking and chemotherapy response. We specifically selected patients with high grade (grade 3) papillary serous ovarian cancer as these tumors are most commonly associated with BRCA-mutations and thus may have a BRCAness phenotype. Again, in that 47-patient cohort, the BRCAness profile was capable of discriminating between long and short median overall survival (OS); the patients with BL and NBL profiles had median OS times of 98 months and 22 months, respectively (log-rank P = 0.07). The ability to achieve a clinical remission for the BL and NBL groups at the end of chemotherapy was 93.3% compared with 68.8% (two-sided Fisher's exact P=0.08).

In the complete dataset of 117 patients from all four different institutions we found that the patients with the BL profile had a better median overall survival, better response to chemotherapy (based on achievement of complete clinical response) and lower risk of death in multivariate analysis compared to patients with NBL profile. These results are summarized in the Table 3 and Figure 6 below. These data represent an important independent validation step for our profile which appears to correlate with outcome and response to chemotherapy in several different datasets including patients from multiple institutions, i.e. Duke, BIDMC, Cedars Sinai and Memorial Sloan Kettering Cancer Center.

	BL Profile	**NBL Profile**	**P value**
Median Overall Survival (months)	98	34	0.001
Response to platinum Chemotherapy (% Complete Response)	91.4%	71.9%	0.027
Hazard Ratio of Death (multivariate analysis)	1	2.82 (95% CI 1.46-5.44)	0.002

Table 3. Association of BRCAness profile with survival and platinum response in all 117 patients

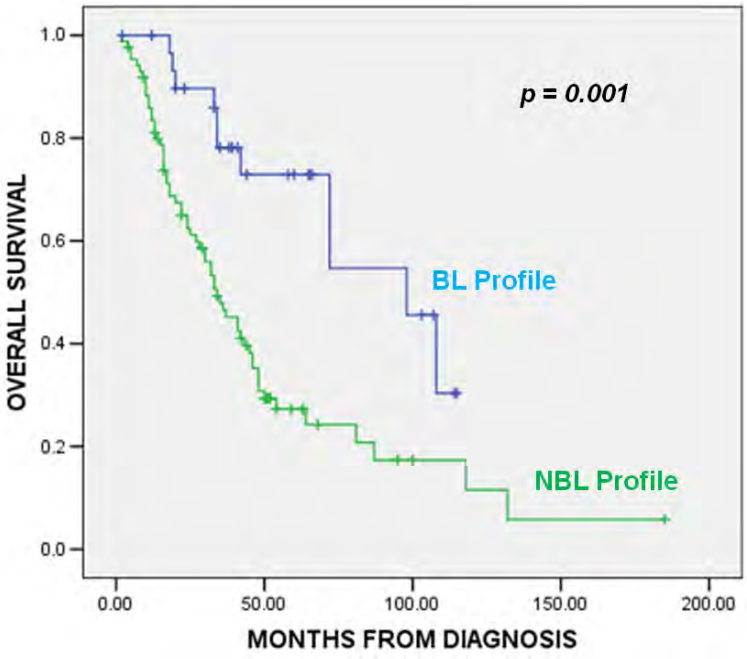

Figure 6. Association of BRCAness profile with OS in all 117 patients. Median OS for BL and NBL patients was 98 and 34 months, respectively (*P* = 0.001).

In conclusion, these data support our hypothesis that the BRCAness profile is associated with survival as well as with clinical response to platinum among patients with sporadic disease. Sporadic tumors with the BRCAlike (BL) profile were associated with increased overall survival as well as platinum sensitivity as evaluated using several metrics of platinum response including achievement of complete response after platinum chemotherapy, disease free survival and percentage of patients who are disease free at 4 months, 6 and 18 months. Of note, multivariate analysis which included the BRCAness profile, age, stage, grade, histology, and debulking status, demonstrated that the profile maintained an independent association with DFS and OS. Furthermore, independent validation of BRCAness profile in other datasets showed that it maintained its association with survival and platinum responsiveness.

Aim 3. Evaluate whether the compounds identified by the Connectivity Map can reverse PARP inhibitor resistance in vitro, and to investigate the mechanism for this effect **(months 1-30)**

We have assessed the IC50 of carboplatin and 17-allylamino-geldanamycin (17-AAG) in the C2-8 and C2-14 clones of the Capan-1 cell line and of the 36M2 cell line. C2-8 is a Capan-1 clone which is PARP inhibitor (PARPi) sensitive, platinum resistant and carries the BRCA2.6174delT mutant allele. As a result, BRCA2 is non-functional in C2-8 cells and homologous recombination is impaired. Conversely, C2-14 is a Capan-1 clone which is PARPi and platinum resistant and carries the original mutant BRCA2.6174delT allele and another BRCA2 allele with the 6174delT sequence and secondary mutations in BRCA2 which restore BRCA2 function. As a result, BRCA2 is functional in C2-14 and homologous recombination is impaired. The 36M2 human EOC cell line has been derived in our lab from serial passage of ovarian serous carcinoma cells in nude mice and exhibits clinical and histologic characteristics similar to papillary serous EOC in humans.

36M2, C2-8 and C2-14 cells have been treated for 24 hours with various concentrations of carboplatin (0μM-150μM) and 17-AAG (0.25μM-10μM). Cell death was appreciated in interval time points from 24 hours to 140 hours and the IC50s for carboplatin and 17AAG for each cell line are summarized in Table 4 below:

IC50	Carboplatin (μM)	17-AAG (μM)
36M2	12	0.30
C2-14	70	0.75
C2-8	55	0.6

Table 4. IC50 of 36M2, C2-14 and C2-18 cell lines for carboplatin and 17-AAG

We are in the process of evaluating the IC50s of the other agents in these cell lines but our preliminary results suggest that 36M2, C2-14 and C2-8 cells have differential platinum and 17-AAG sensitivity, thereby allowing us to evaluate the effects of 17-AAG (which has been identified by the Connectivity Map) in cellular systems of diverse chemosensitivity.

Aim 4. Determine the reproducibility of the BRCAness profile when using the DASL mRNA assay in a cohort of FFPE ovarian cancer specimens with known clinical outcome and platinum responsiveness **(months 1-42)**

We have already isolated RNA from formalin fixed paraffin-embedded (FFPE) specimens of 30 sporadic patients treated at BIDMC with known outcome and platinum response data. FFPE blocks were analyzed to identify regions containing relevant tumor cells and four to six 1 mm cores were taken from those regions using a microtome available at BIDMC Histology Core facility. Cores were placed in Eppendorf tubes and paraffin was removed by sequential treatment with Xylene and ethanol. Proteins were digested with Proteinase K and total RNA extraction was performed using the Qiagen RNeasy FFPE protocol according the manufacturer's protocol. RNA quantity was measured using the Nanodrop spectrophotometer and quality was assessed using the Agilent Bioanalyzer 2100. We tested several protocols for RNA extraction but the Qiagen RNeasy FFPE protocol performed extremely well in all of our quality checks. In this regard, we used quantitative RT-PCR to measure representation of various genes (NQO1, SOD2, GPX3) to assess the level of intact message present. Further analysis of the quality of the RNA was provided by repeat assays of separate preparations from the same block.

RNA was reverse transcribed into complementary DNA (cDNA) with Promega's Reverse Transcription System according to the manufacturer's protocol. Real-time PCR was set up with Roche Universal Probe Library hydrolysis probes and Probes Master reagents and amplification was performed in triplicate on the LightCycler 480 (Roche, Indianapolis, IN). The primers used for genes NQO1, SOD2, GPX3 were designed using ProbeFinder online tool from Roche. Our RNA yield was 25-50 μg per sample, and 2.5 μg were used for each RT PCT reaction. We also run pairs of frozen/paraffin tumor tissue samples and all mRNAs were detected as expressed in all samples. In addition, the Spearman correlation coefficients for the various mRNA targets in the frozen-paraffin paired samples were highly statistically significant ($p<0.01$). Finally, correlation coefficients for replicates (separate preparations from the same block) were also highly statistically significant ($p<0.01$).

In conclusion, these data demonstrate that we can extract high quality RNA from formalin fixed paraffin embedded ovarian cancer sections and that the detection and

quantitation obtained is highly concordant with that obtained using frozen tissue. We are currently working on isolating RNA from more sporadic patients so that we can proceed with the whole genome DASL arrays.

KEY RESEARCH ACCOMPLISHMENTS

- BRCAness profile is able to track diverse molecular mechanisms that cause defective homologous recombination (HR) in the absence of BRCA1 or BRCA2 mutations
- BRCAness profile is associated with platinum sensitivity in vitro
- BRCAness profile is associated with survival in patients with sporadic disease
- BRCAness profile is associated with clinical sensitivity to platinum as evaluated using various metrics of platinum response in sporadic patients
- High quality RNA from formalin fixed paraffin embedded ovarian cancer sections can be extracted and its detection and quantitation is highly concordant with that obtained from frozen tissue

REPORTABLE OUTCOMES

1. The following manuscripts have been published during this funding period:

- Konstantinopoulos PA, Spentzos D, Karlan BY, et al. Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. J Clin Oncol;28:3555-61.
- Konstantinopoulos PA, Cannistra SA, Fountzilas H, et al. Integrated analysis of multiple microarray datasets identifies a reproducible survival predictor in ovarian cancer. PLoS One;6:e18202

2. The following abstract (please see Appendix for full abstract) was selected for presentation in the 2011 National Cancer Institute (NCI) Translational Science Meeting "From Molecular Information to Cancer Medicine", July, 2011, Washington, DC.

- Konstantinopoulos PA et al. A gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and outcome in epithelial ovarian cancer

3. The microarray dataset with the generated RNA expression data of the sporadic patients has been deposited in the publicly available Gene Expression Omnibus (GEO), http://www.ncbi.nlm.nih.gov/geo/, Accession number GSE19829.

4. During this funding period I was promoted to Assistant Professor of Medicine at Harvard Medical School.

CONCLUSION

Our results thus far support the hypothesis that the 60-gene profile may be associated with several surrogates of BRCAness such as improved outcome and responsiveness to chemotherapy. Specifically, BRCAness profile is associated with platinum sensitivity in vitro and in vivo, and is associated with survival in patients with sporadic disease. Furthermore, our profile can track diverse molecular mechanisms that cause defective homologous recombination (HR) in the absence of BRCA1 or BRCA2 mutations. Our current studies are focusing on assessing the correlation of the profile with PARP inhibitor responsiveness, and evaluating the reproducibility of the profile using the DASL mRNA assay in formalin fixed, paraffin-embedded ovarian cancer specimens.

REFERENCES

1. D'Andrea AD. Susceptibility pathways in Fanconi's anemia and breast cancer. N Engl J Med;362:1909-19.
2. Elkon R, Rashi-Elkeles S, Lerenthal Y, et al. Dissection of a DNA-damage-induced transcriptional network using a combination of microarrays, RNA interference and computational promoter analysis. Genome Biol 2005;6:R43.
3. Furuta S, Wang JM, Wei S, et al. Removal of BRCA1/CtIP/ZBRK1 repressor complex on ANG1 promoter leads to accelerated mammary tumor growth contributed by prominent vasculature. Cancer Cell 2006;10:13-24.
4. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer 2006;6:813-23.

5. Liu H, D'Andrade P, Fulmer-Smentek S, et al. mRNA and microRNA expression profiles of the NCI-60 integrated with drug activities. Mol Cancer Ther;9:1080-91.

6. Konstantinopoulos PA, Spentzos D, Karlan BY, et al. Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. J Clin Oncol;28:3555-61.

7. Konstantinopoulos PA, Cannistra SA, Fountzilas H, et al. Integrated analysis of multiple microarray datasets identifies a reproducible survival predictor in ovarian cancer. PLoS One;6:e18202.

APPENDICES

Supplement Table 1. Clinical and Pathological Characteristics of patients

Characteristic		Sequenced Cohort[a] (n=35)		Non-Sequenced Cohort[b] (n=35)		Combined Cohort[c] (n=70)	
		No.	%	No.	%	No.	%
Age	Median (range)	62.3 (44-89)		58.7 (39-80)		60.5 (39-89)	
Grade	1-2	4	11.4	6	17.1	10	14.3
	3	31	88.6	29	82.9	60	85.7
Histology	Serous	34	97.1	31	88.6	65	92.9
	Clear Cell	0	0	2	5.7	2	2.9
	Endometrioid	1	2.9	2	5.7	3	4.3
Stage	2	2	5.7	1	2.9	3	4.3
	3	28	80	29	82.9	57	81.4
	4	5	14.3	5	14.3	10	14.3
Debulking Status[d]	Optimal	27	79.4	22	62.9	49	71
	Suboptimal	7	20.6	13	37.1	20	29

[a] All 35 patients underwent sequencing and were wildtype for BRCA-1 and -2.

[b] Not sequenced but enriched for sporadic disease on the basis of the following: no family history of ovarian cancer, no family history of breast cancer under the age of 50 years, no family history of more than 1 breast cancer at any age, and not of Ashkenazi Jewish ethnicity.

[c] All patients received first line platinum-based chemotherapy

[d] Debulking status was unknown for 1 patient. Optimal, less than or equal to 1 cm.; suboptimal, greater than 1 cm.

Abstract selected for the 2011 National Cancer Institute (NCI) Translational Science Meeting "From Molecular Information to Cancer Medicine", July, 2011, Washington, DC

PURPOSE: To define a gene expression profile of BRCAness that correlates with responsiveness to platinum and PARP inhibitors.

METHODS: A publicly available microarray dataset including 61 patients with epithelial ovarian cancer (EOC) with either sporadic disease or BRCA-1/-2 mutations was used for development of the BRCAness profile. Correlation with platinum responsiveness was assessed in platinum sensitive and resistant tumor biopsy specimens from 6 patients with BRCA germline mutations. Association with PARP inhibitor responsiveness was assessed in 2 PARP inhibitor-resistant and 2 -sensitive Capan-1 clones. The BRCAness profile was applied to 70 patients enriched for sporadic disease in order to assess its association with outcome.

RESULTS: A 60-gene diagonal linear discriminant predictor distinguished BRCA-like (BL) from non-BRCA-like (NBL) tumors with 94% accuracy, as assessed by leave-one-out cross-validation (1000 random permutations test $p<0.001$). The BRCAness profile accurately distinguished between platinum sensitivity and resistance in 8 out of 10 tumor specimens, and between PARP-inhibitor sensitivity and resistance in 4 out of 4 Capan-1 clones. When applied to the 70 patients who were enriched for sporadic disease, patients with the BL profile had improved DFS (34 months vs 15 months, log-rank $P = 0.013$) and OS (72 months vs 41 months, log-rank $P = 0.006$) compared to NBL patients, respectively. The BRCAness profile maintained independent prognostic value in multivariate analysis, controlling for other known prognostic factors such as age, stage, grade, histology, and debulking status.

CONCLUSION: The BRCAness profile correlates with responsiveness to platinum and PARP inhibitors and identifies a subset of sporadic patients with improved outcome. Use of this profile may ultimately permit identification of sporadic EOC patients who may benefit from PARP inhibitor therapy.